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Sequential Dehalogenation of Chlorinated Ethenes

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16. ABSTRACT

Reductive dehalogenation of tetra- and tri-chloroethene to cis- and trans-1,2-dichloroethene in microcosms simulating ground water environment has previously been demonstrated. In this study, anoxic microcosms containing organic sediment and water were spiked to contain 5 mg/L of one of the following compounds: 1,1-dichloroethene (1,1-DCE), cis-1,2-dichloroethene (CIS), or trans-1,2-dichloroethene (TRANS). After incubation in the dark at 25°C for up to six months, contents were analyzed by gas chromatography, and verified by GC/MS in an attempt to identify sequential steps in the transformation process. Vinyl chloride (VC) was produced after 1 to 2 weeks of incubation in all spiked microcosms, but none was observed in sterile and unspiked controls. Chloroethane (CE) was produced only in microcosms spiked with CIS, indicating isomer specificity and the occurrence of mechanisms other than reductive dechlorination. Kinetics parameters associated with the microbial dehalogenation of 1,1-DCE, CIS, and TRANS were calculated.

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JOURNAL ARTICLE

Sequential Dehalogenation of Chlorinated Ethenes

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■ Reductive dehalogenation of tetra- and trichloroethene to *cis*- and *trans*-1,2-dichloroethene in microcosms simulating groundwater environment has previously been demonstrated. In this study, anoxic microcosms containing organic sediment and water were spiked to contain 5 mg/L of one of the following compounds: 1,1-dichloroethene (1,1-DCE), *cis*-1,2-dichloroethene (CIS), or *trans*-1,2-dichloroethene (TRANS). After incubation in the dark at 25 °C for up to 6 months, contents were analyzed by gas chromatography and verified by gas chromatography/mass spectrometry in an attempt to identify sequential steps in the transformation process. Vinyl chloride (VC) was produced after 1–2 weeks of incubation in all spiked microcosms, but none was observed in sterile and unspiked controls. Chloroethane (CE) was produced only in microcosms spiked with CIS, indicating isomer specificity and the occurrence of mechanisms other than reductive dechlorination. Kinetic parameters associated with the microbial dehalogenation of 1,1-DCE, CIS, and TRANS were calculated.

Introduction

Previous studies of transformations of chlorinated alkenes in microcosms simulating underground environments (*1*, *2*) indicated that tetrachloroethene (PCE) and trichloroethene (TCE) undergo reductive dehalogenation to form *cis*- and *trans*-1,2-dichloroethene (CIS and TRANS, respectively). This was recently verified with TCE isotopically labeled with one ^{13}C atom (*3*). Further transformation to vinyl chloride was indicated (*4*) but not proven. It was reported (*2*, *4*) that microcosms spiked to contain 5 mg/L of a chlorinated alkene produced compounds with one less chlorine than the parent substrate in quantities less than 10% of the original compound, in 8 weeks of incubation. This means that if *cis*- and *trans*-1,2-dichloroethene were transformed to vinyl chloride, the resulting concentration of vinyl chloride was too small for detection by the methods used (*1*, *4*).

Horowitz et al. (*5*) and Suflita et al. (*6*) reported sequential reductive dehalogenation of halogenated benzoates with 100% of the dichlorinated benzoates transformed to monochlorinated benzoates in approximately 1 month.

Bouwer and McCarty (*7*, *8*) studied the biotransformation of several 1- and 2-carbon halogenated aliphatic compounds under methanogenic and denitrification conditions. They observed that removal of chlorine by bio-oxidation or hydrolysis can occur simultaneously with reductive dehalogenation, thus causing a greater confusion in determining the mechanistic steps involved in the complete removal of halogenated compounds.

Chlorinated ethenes transform very slowly, and apparently with several simultaneous removal reactions. All of the intermediate products of biotransformation between PCE and vinyl chloride, including vinyl chloride itself, that were observed in laboratory studies have been found in groundwater. The processes involved and rates at which these changes occur are important in assessing risk from use of affected waters. This study was made to further elucidate the behavior and fate of these toxic environmental contaminants by examining each intermediate step of the transformation of PCE to VC.

The specific objective of this research was to study the biotransformation of CIS, TRANS, and 1,1-DCE to vinyl chloride and to measure the rate of depletion of these substrates in microcosms simulating groundwater environments.

Experimental Procedures

Chemicals. 1,1-Dichloroethene (99%) (1,1-DCE), *cis*-1,2-dichloroethene (97%) (CIS), and *trans*-1,2-dichloroethene (98%) (TRANS) were purchased from Aldrich Chemical Co., Milwaukee, WI. Vinyl chloride (0.2 mg/mL methanol) (VC) was obtained from Supelco, Inc., Bellefonte, PA. Chloroethane (CE) was purchased from Eastman Chemical Co., Rochester, NY.

Preparation of Microcosms. Natural organic sediment collected from the Everglades, a graminoid wetland that is the recharge basin for the Biscayne Aquifer in southern Florida, was used to construct microcosms. Muck samples from two sites in the same area were obtained: site "A", which was the bottom of a shallow canal, and site "B", which was near the surface. Sites previously uncontaminated by chlorinated organic compounds were chosen for sediment sources to prevent selection of adapted microorganisms, which may yield different and variable results depending on quantity, nature, and age of contaminant.

Each sediment sample was thoroughly mixed and passed through a 6.34-mm sieve and then weighed (wet) into 50-mL septum bottles. Care was taken to prepare microcosms as uniformly as possible. The sediments were used in their natural state. Average dry weight was determined for purposes of comparison and to determine uniformity on a separate set of subsamples and amounted to 4 g dry weight per 50-mL bottle. After addition of sediment, the bottles were completely filled with water taken from the sample site. The water was purged with nitrogen prior to use to eliminate any highly volatile contaminants and to purge oxygen entrapped by sampling. Microcosms were prepared in sets that included the following controls: sterile, no-spike, and distilled water. Distilled water controls contained the organic sediment, but nitrogen-purged distilled water was substituted for site water. Sterile controls were prepared by autoclaving the organic sediments and the site water for 20 min on two consecutive days. After the materials were cooled, microcosms were constructed under aseptic conditions and sealed with sterile Teflon-lined septa, preventing headspace formation.

Spiking solutions were prepared in 50-mL serum bottles that were autoclaved along with the distilled water, magnetic stirrers, pipets, and the microsyringe used to prepare solutions. Accurately measured amounts of the three compounds, CIS, TRANS, and 1,1-DCE, were injected into separate, sterile bottles containing 50 mL of sterile, nitrogen-purged water and allowed to stir overnight. The final concentration of each compound in the spiking solutions was 500 mg/L. Half milliliter of each solution was spiked into each 50-mL microcosm to yield a final concentration of 5 mg/L in the microcosms. The microcosms were spiked 2 weeks after construction to allow equilibration and oxygen depletion to occur inside the test and control bottles and thus simulate the original conditions of the sample site. All microcosms and controls were al-

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lowed to incubate in the dark at 25 °C for measured time periods of up to 6 months. Repeated sampling of a single microcosm in preliminary studies caused contamination and a change in the volume of the contents and introduced a gas phase (headspace). For this reason, duplicate microcosms were constructed as described above, for each scheduled test period, so that each microcosm was used only once in an analysis. Replicate microcosms were provided for concurrently measuring pH and redox potential (Eh). Although the microcosms were prepared homogeneously, variability of activity was accounted for by calculating the mean of replicate runs.

Sterility of sterile controls and spiking solutions was determined by streaking these materials on plates of R_2A medium (*2*, *9*), incubating the plates, and observing them for growth.

Instrumentation. A Tracor Model 222 gas chromatograph with a 244 cm × 2.5 mm i.d. stainless-steel column, packed with 60/80 mesh Tenax GC, and a Hall electrolytic conductivity detector, Model 700, were employed for analysis. Nitrogen carrier gas at 40 mL/min and hydrogen reaction gas at 50 mL/min were supplied. The column oven was programmed to hold isothermal for 6 min at 40 °C while 5 mL of microcosm contents or standards were purged with N_2 directly on the head of the column (*10*). The column temperature was then increased from 40 to 220 °C at 8 °C/min. The temperature of the detector was kept at 850 ± 20 °C. The three isomers were successfully separated under the stated working conditions as follows: 1,1-DCE at 15.03 min of retention time and TRANS and CIS at 16.21 and 17.53 min, respectively.

Gas chromatography/mass spectrometry (GC/MS) data were obtained on a Finnigan 4500 GC/MS system interfaced to a Tekman LSC-2 purge-and-trap system. The gas chromatographic column was a 6 ft × 2 mm i.d. 0.2% Carbowax 1500 on 80/100 Carbopack B. The data system was standardized for the dichloroethenes and the purgeable gases (vinyl chloride, bromoethane, chloromethane, and chloroethane). An internal standard mix of fluorobenzene and *p*-bromofluorobenzene at the 25 μg/L level was added to each sample on the basis of volume of extractant water available.

Redox potentials (Eh) and pH were measured with a Corning Model 7 meter by using a Corning glass electrode for pH measurements and a Fisher platinum combination electrode for redox measurements. For pH measurements the instrument was calibrated at pH 4 and 7 with Scientific Products reference buffer solutions. For Eh measurements the instrument was calibrated at 180 and 0.0 mV by using pH 4 and 7 buffer solutions supersaturated with hydroquinone crystals.

Standard Solutions. Stock aqueous solutions of 1,1-DCE, CIS, and TRANS were prepared at 500 mg/L (ppm), by volumetric dilution, similar to the spiking solutions. Working standard of the five chlorinated compounds, 1,1-DCE, CIS, TRANS, VC, and CE, were prepared by diluting aliquots of the stock solutions with water and stirring overnight to achieve solutions of the desired concentrations. All bottles were wrapped in aluminum foil to avoid photodecomposition and kept at 4 °C. The system was checked daily against standard solutions and was recalibrated when the deviation was greater than 3%.

Results and Discussion

Evidence for Dehalogenation. The organic compounds produced when 1,1-DCE, CIS, and TRANS were incubated with oxygen-depleted sediment were identified by gas chromatography/mass spectrometry. Vinyl chloride was detected in all microcosms (Figures 1–3). VC was not detected in the no-spike and sterile controls. A peak identified as chloroethane was observed to increase with

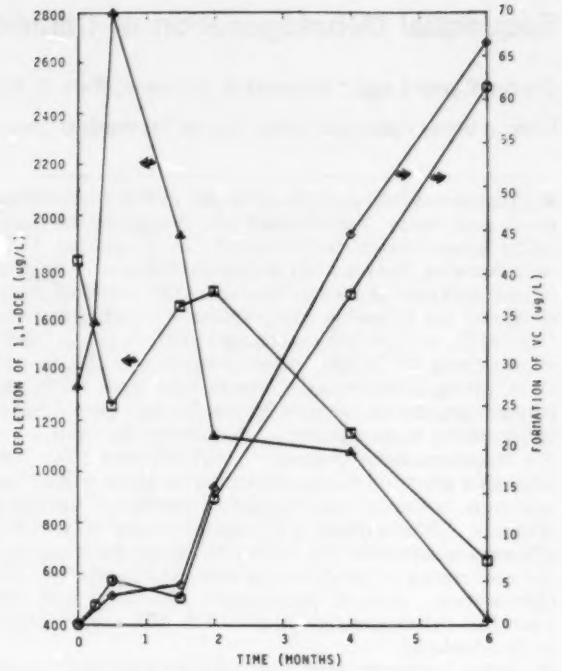

Figure 1. Patterns of anaerobic degradation of 1,1-DCE by sediment and water microcosms. (Δ) Depletion of 1,1-DCE in sediment and water from site B. (□) Depletion of 1,1-DCE in sediment and water from site A. (◊) Formation of VC in sediment and water from site B. (O) Formation of VC in sediment and water from site A.

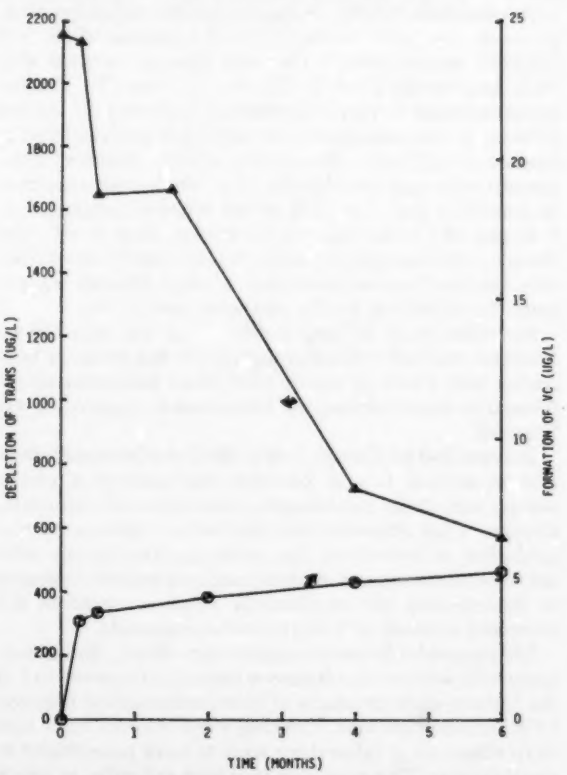

Figure 2. Pattern of anaerobic degradation of TRANS in sediment and water microcosms. Site A muck sample. (Δ) Depletion of TRANS. (O) Formation of VC.

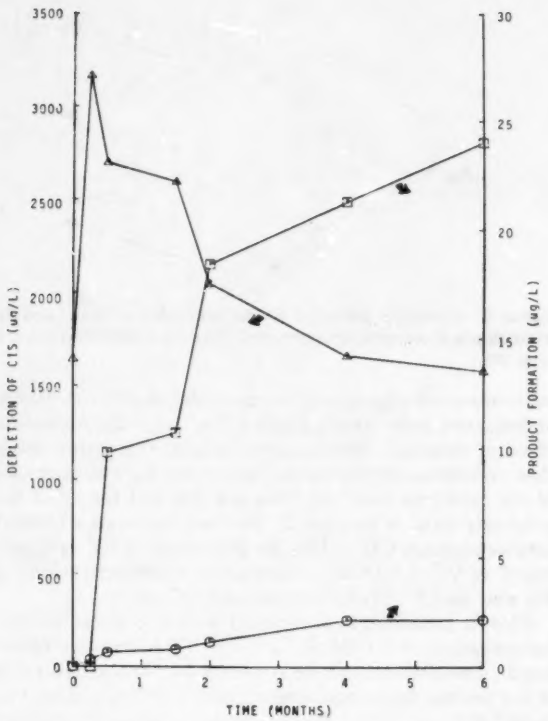

Figure 3. Pattern of anaerobic degradation of CIS in sediment and water microcosms. Site A muck sample. (△) Depletion of CIS. (□) Formation of CE. (○) Formation of VC.

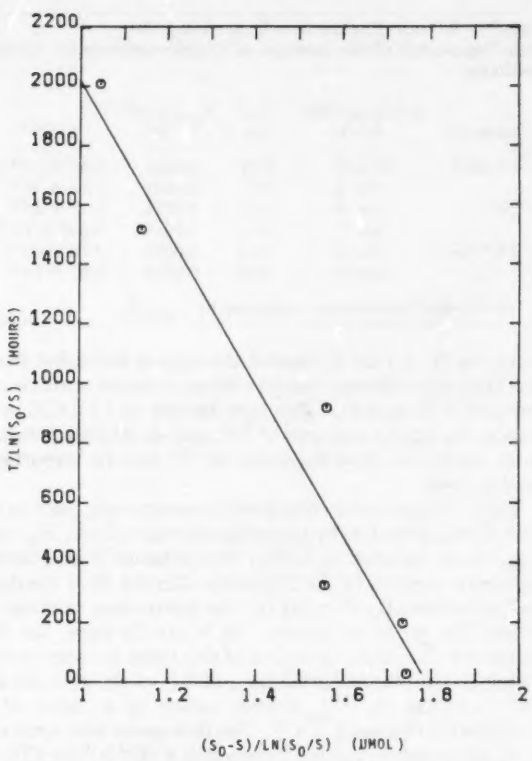

Figure 4. Michaelis–Menten fit of the data (eq 1) from the depletion of TRANS in sediment and water from site B.

time in all microcosms spiked with CIS (Figure 3). Samples spiked with 1,1-DCE and TRANS did not produce CE, nor did the sterile microcosms spiked with CIS. Analyses were made only for the intermediate compounds VC and CE and not for the complete mineralization of the spike. Only the intermediate steps were sought to demonstrate the stepwise nature of the transformation process.

Dechlorination of the three compounds under study did not occur in sterilized microcosms. In microcosms spiked with 1,1-DCE and TRANS (Figures 1 and 2), only the formation of vinyl chloride was observed. When CIS was the parent substrate (Figure 3), however, CE also was produced. In microcosms spiked with CIS (Figure 3), the concentration of VC remained low and nearly constant after 2 weeks of incubation, while chloroethane increased slowly. Microcosms spiked with TRANS (Figure 2) showed production of VC similar to the transformation of CIS; i.e., low and nearly constant concentration after 2 weeks, but CE was not produced in this case.

Figures 1 and 3 show that the depletion of the parent substrate was frequently preceded by an apparent initial increase in concentration. This was caused by an initial physical equilibration time and adaptation of the microbiota to the microcosm and assimilation of the introduced substrate. The microcosms were shaken vigorously upon spiking to distribute the chlorinated substrate. As the microcosm contents were allowed to settle in the dark at room temperature, most of the added organic compound was trapped, and some was adsorbed in the interstices of the solid phase, i.e., muck. Equilibrium between solid and liquid phases was established in about 2-weeks incubation time. Other authors have reported lag periods of up to 6 months (*5*), caused by the adaptation of the microbiota to utilize one specific substrate.

The inconsistency of this initial equilibration time made it impossible to measure the initial velocities that are valuable in calculating the true order of reactions.

Figure 3 is a typical plot of a consecutive reaction where the concentration of the intermediate (VC) rose to a maximum and then decreased or stayed constant, and the concentration of the last product (CE) rose to a maximum. However, VC is not believed to be an intermediate species between CIS and CE because chloroethane was not observed in samples spiked with 1,1-DCE and TRANS where VC was detected.

First-order kinetic models were attempted to depict the decay of 1,1-DCE, CIS, and TRANS. In every case, however, we found that semilogarithmic plots of the data deviated from linearity usually after 3–4 months of incubation.

Horowitz et al. (*5*) observed that when 3-chlorobenzoate was the initial substrate, the data followed first-order depletion kinetics. If the parent substrate was 3,5-dichlorobenzoate (*6*), the dichlorinated substrate competitively inhibited the dehalogenation of the monochlorinated substrate, causing a deviation from first-order kinetics. These authors (*6*) found that dehalogenation of mono- and dichlorinated benzoates exhibited Michaelis–Menten kinetics. Good linearity was obtained when our data was fitted to the linearized form of the Michaelis–Menten equation which was also used by Suflita et al. (*6*)

$$t/[\ln (S_0/S)] = (1/V)[(S_0 - S)/\ln (S_0/S)] + K_m/V \quad (1)$$

where t is time, S_0 is the initial substrate concentration, S is the substrate concentration at time t, V is the maximum rate of substrate depletion, and K_m is the half-saturation constant. Michaelis–Menten kinetics only describes bacterial substrate when this process is unlinked to growth of microbial biomass.

Figure 4 shows the Michaelis–Menten fit of the data for the depletion of TRANS in microcosms with sediment

Table I. Kinetic Parameters Describing the Dehalogenation of the Isomers of Dichloroethene by Anoxic Sediment

substrate	dehalogenation muck	K_m, μm	V_{max}, μmol L^{-1} h^{-1}	k_1, h^{-1} [a]
1,1-DCE	site B	43.4	0.0155	3.57×10^{-4}
	site A	36.7	0.0061	1.67×10^{-4}
CIS	site B	65.3	0.0214	3.28×10^{-4}
	site A	41.8	0.0036	0.853×10^{-4}
TRANS	site B	35.8	0.0078	2.19×10^{-4}
	site A	29.6	0.0058	1.97×10^{-4}

[a] First-order rate constant obtained by V_{max}/K_m.

from site B. 1,1-DCE showed the largest deviation from the Michaelis–Menten model with correlation coefficients between 0.75 to 0.91. This may be due to 1,1-DCE producing the largest amounts of VC, and an inhibition factor from the consecutive depletion of VC may be important in this case.

Table I summarizes the kinetic parameters associated with dehalogenation by the sediment microflora. K_m and V_{max} values reported in Table I were attained from a linear regression analysis of the Michaelis–Menten fit of the data and subsequently divided by the microcosm volume to obtain the reported values. As it can be seen, the K_m values for the dehalogenation of the three isomers in the tested environment varied by a factor of about 2 (30–65 μM), whereas the V_{max} values varied by a factor of 6 (0.0036–0.0214 μmol L^{-1} h^{-1}). The first-order rate constant of dehalogenation ranged from 0.853×10^{-4} h^{-1} for CIS in sediment to 3.57×10^{-4} h^{-1} for 1,1-DCE in site "B" sediment, indicating a very slow rate of dehalogenation.

pH of all microcosms was measured and found to remain constant at 7.0 ± 0.5. Redox potential data (Eh) changed drastically in the first 2 weeks of incubation while equilibrium was attained, after which time it remained almost constant between −20 and −130 mV.

Not all the substrate that was depleted was transformed to VC (Figures 1–3), indicating that mechanisms of transformation other than reductive dechlorination were taking place. Transformation of substrate by biooxidation (*7*) or hydrolysis (*8*) are possible removal mechanisms. The appearance of CE (Figure 3) in microcosms spiked with CIS indicates isomer specificity and a different pathway of transformation in the sequential dechlorination.

Figure 5 shows a summary of transformations that may occur to tetra- and trichloroethene in oxygen-depleted sediment. Steps a–c were reported previously (*2*). Step d was suspected to occur but never reported because of possible interference from contaminants (*2*). Recently it was reported (*3*) that this isomer did not result from the biotransformation of isotopically labeled trichloroethene. Steps e–h are reported in this paper. It is not known whether steps e and f occur independently or are interrelated.

Depletion of these substrates, similar to that reported for trichloroethylene (*2*), required longer periods of time than other chlorinated alkanes reported by different authors (*5–8*). Most probably the slow biotransformation of such compounds caused Bouwer et al. (*11*) to report observing no appreciable anaerobic degradation of tetra- and trichloroethylene. Figures 1–3 show that only 50–80% of 1,1-DCE, CIS, and TRANS was depleted in 6 months of incubation.

Conclusions

This study has shown that 1,1-DCE, CIS, and TRANS, subjected to the indigenous microbiota of uncontaminated

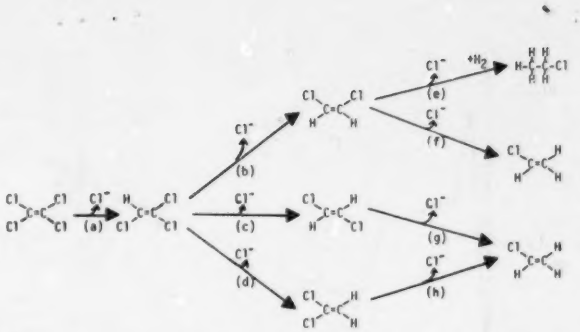

Figure 5. Summary pathways for dechlorination of tetra- and trichloroethene in anerobic environments. Steps a–c from Parsons and Lage (*2*).

environmental organic sediments, under anoxic conditions, undergo reductive dechlorination leading to the formation of vinyl chloride. Mechanisms of transformation other than reductive dechlorination also occur because the sum of the products observed does not account for all of the substrate that is removed. Isomers undergo different tranformations; CIS led to the formation of CE and only traces of VC; 1,1-DCE yielded greater concentrations of VC and no CE; TRANS produced VC only.

Kinetic parameters associated with the microbial dehalogenation of 1,1-DCE, CIS, and TRANS were calculated. The first-order rate constant k_1, for the depletion of the parent substrate ranged from 0.853×10^{-4} to 3.57×10^{-4} h^{-1}.

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Registry No. 1,1-DCE, 75-35-4; CIS, 156-59-2; TRANS, 156-60-5; VC, 75-01-4; CE, 75-00-3; PCE, 127-18-4; TCE, 79-01-6.

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